

## Protein-Lipid Interaction in Rhodopsin Recombinant Membranes as Studied by Protein Rotational Mobility and Lipid Alkyl Chain Flexibility Measurements<sup>1</sup>

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Protein rotational mobility was measured by saturation transfer ESR of a covalently attached spin label, and lipid alkyl chain flexibility by conventional ESR of 5-doxyl and 16-doxyl stearate in rhodopsin-dimyristoylphosphatidylcholine recombinants as a function of temperature and of the protein content. Rhodopsin caused some reduction of the lipid alkyl chain flexibility, the degree of which gradually increased with the protein content. In the protein-rich recombinants, a strongly immobilized component appeared and its relative intensity increased with the protein content and at temperatures lower than that of the phospholipid phase transition. This component was ascribed to lipid spin labels entrapped in the protein clusters.

The rotational mobility of rhodopsin changed discontinuously at the host lipid phase transition. However, the change in the rotational correlation time was not large. The weakened response to the phase transition was ascribed to residual mobility in the solid phase and remaining aggregation of rhodopsin above the phase transition. The temperature dependence became more and more broadened with the increase of protein content, probably due to greater aggregation in the fluid phase and partly due to increased average viscosity.

Rhodopsin-dielaidoylphosphatidylcholine recombinants were also studied for comparison and some differences in the lipid-protein interaction were noted.

Dynamic protein-lipid interactions are undoubtedly one of the most important problems remaining in membrane molecular biology. This subject has been studied mostly from the viewpoint of lipids

influenced by proteins, and little information has been obtained in systems of proteins affected by lipids. This has been due to the unavailability of experimental methods to measure protein mobility

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Abbreviations: DMPC, dimyristoylphosphatidylcholine; DEPC, dielaidoylphosphatidylcholine; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2'-ethanesulfonic acid; SDS, sodium dodecyl sulfate; DNP, dinitrophenylated; BSA, bovine serum albumin; 5-doxyl and 16-doxyl stearate, *N*-oxyl-4',4'-dimethylloxazolidine derivatives of 5- and 16-ketostearic acid, respectively.

in membranes, in contrast to the variety of techniques available for lipids. Recently, several techniques have been developed for determining protein lateral and rotational mobilities (1-3) and the functional significance of the dynamic properties is under extensive investigation.

The present study has been undertaken to investigate dynamic aspects of protein-lipid interactions from both viewpoints, using recombinant membranes of rhodopsin in well-defined phospholipids. The protein rotational mobility was monitored by saturation transfer ESR spectroscopy of the covalently attached spin label and the lipid alkyl chain flexibility (fluidity) by conventional ESR spectroscopy of dispersed stearate spin labels. Emphasis was laid on the effect of protein concentration upon the phospholipid phase transition.

Rhodopsin was chosen because of its characteristic function as a photoreceptor protein and also because there is a wealth of information on the lateral and rotational mobilities in intact disc membranes (4-7). Preparation of rhodopsin recombinants has also been well described, and morphological as well as lipid motional studies have been carried out (8-10). During the preparation of this article, the results of work along similar lines have been published by Devaux's group (11-13).

#### MATERIALS AND METHODS

All the procedures involving rhodopsin were carried out under dim red light (19 W bulb, deep red filter No. 3, Asanuma Co., Ltd.) or in total darkness.

*Preparation of Rhodopsin Recombinant Membranes*—Cattle rod outer segment membranes were prepared by the method of Papermaster and Dreyer (14). Purified phospholipid-free rhodopsin was obtained by concanavalin A-Sepharose (Pharmacia) column chromatography with 30 mM octyl- $\beta$ -glucoside (Sigma) as a detergent (15, 16). Rhodopsin concentration was determined from the difference in the absorbance at 498 nm ( $\Delta A_{498}$ ) upon bleaching a solubilized sample in octyl- $\beta$ -glucoside + Ammonyx LO (Onyx) in the presence of 0.1 M hydroxylamine. The molar extinction coefficient of rhodopsin was taken as 40,000 at 498 nm and the molecular weight as 40,000 (17). Protein and phospholipid were determined by the

method of Lowry *et al.* (18) and Bartlett (19), respectively. The purified rhodopsin gave an optical purity ( $A_{278}/\Delta A_{498}$ ) of about 1.7 and contained residual phospholipid amounting to about 1 mol per mol of rhodopsin.

DMPC and DEPC were synthesized as described previously (20). Recombinants were prepared using cholate (Wako) as a detergent in most experiments. Phospholipid was solubilized with cholate and mixed with the purified rhodopsin at a desired ratio. The final concentration of cholate was 50-75 mM. The detergent was removed by exhaustive dialysis against solutions containing 5 mM HEPES, 1 mM EDTA, and progressively decreasing concentrations of NaCl (200, 150, 100, 50, and 0 mM) at pH 7.4. The recombinant membranes were recovered by centrifugation at 16,000  $\times g$  for 45 min and washed with 65 mM NaCl buffered at pH 6.8 with 10 mM HEPES. Unless otherwise mentioned, the following experiments were carried out in this buffer.

The recovery yield of rhodopsin was estimated at  $88 \pm 5\%$  from the optical purity. The protein was pure on SDS-polyacrylamide gel electrophoresis. Linear density gradient (15-60% w/w sucrose) ultracentrifugation at 160,000  $\times g$  for 5 h gave a very sharp band at a position corresponding to the protein-lipid ratio of recombinant vesicles. Possible formation of lysolecithin was checked by thin layer chromatography developed with  $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{CH}_3\text{COOH}$  (65:35:4, by vols). No spot other than DMPC was observed with molybdenum phosphate.

In some experiments, recombinants were prepared using dodecyltrimethyl ammonium chloride as the detergent, as described previously (8).

*Spin Labeling*—Rhodopsin in recombinants was spin-labeled with a maleimide derivative, *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxy) maleimide, synthesized and purified by recrystallization in this laboratory. In most experiments, recombinants were pretreated with 1 mM *N*-ethylmaleimide (Nakarai) at 0°C for 45 min and washed twice by centrifugation. The recombinants were then made to react with the maleimide spin-label reagent at 0°C for 40 h and washed seven times. The number of attached spin labels was estimated by double integration of the ESR spectrum to be 1.1 labels per rhodopsin. According to Fung and Hubbell (21), the maleimide spin label should be

on the carbohydrate-carrying segment of rhodopsin. For comparative studies, recombinants were first made to react with the maleimide spin-label reagent at 0°C for 45 min and then with *N*-ethylmaleimide. The number of attached spin-labels was 1.2 per mol of rhodopsin in this case. The spin label should be attached to the retinal-carrying segment (21). In some experiments, rhodopsin in recombinants was spin-labeled as described previously (7), by reaction with the maleimide reagent at a molar ratio of 1 : 100 at 0°C for 8 h.

For spin-labeling with stearate, the bottom of a test tube was coated with a thin film of spin-labeled stearate and the recombinant suspension was poured into the tube at a molar ratio of spin-label to phospholipid of about 1 : 100. The mixture was kept overnight at 0°C, transferred to another tube, and washed by centrifugation. Two kinds of spin labels, 5-doxyl and 16-doxyl stearates, were used.

**Cross-Linking of Rhodopsin Molecules in Recombinant**—In order to investigate whether the spin label motion reflects the rotational motion of rhodopsin as a whole, rhodopsin molecules were cross-linked with glutaraldehyde or with antibodies. The chemical cross-linking was done by treating recombinants with a 10<sup>4</sup>-fold excess of 2.5% glutaraldehyde (Ladd, 70% w/w) at 0°C overnight. For further cross-linking, BSA was added to the glutaraldehyde-treated recombinant at 0°C at a final concentration of 5 mg/ml and left overnight. For cross-linking with antibodies, the recombinants were first dinitrophenylated with 1.2 mM dinitrophenylfluorobenzene at 0°C overnight and washed. Anti DNP-BSA antisera produced in goat (about 8 mg, Miles) was added to the dinitrophenylated rhodopsin (4 mg), then the mixture was incubated at 31°C for 2 h, and washed three times by centrifugation. The pellet was resuspended and incubated with anti goat-IgG rabbit antisera (about 4 mg, Miles) at 31°C for 2 h. The antibody-bound recombinants were further cross-linked with glutaraldehyde and washed twice. Further cross-linking with BSA was also carried out as described above.

**ESR Measurements**—Conventional and saturation transfer ESR spectra were measured on a JEOL FE-2X spectrometer with a variable temperature accessory. A cylindrical cavity (TE<sub>011</sub>

mode) was used. The sample tube was a quartz capillary with an inside diameter of 0.59 mm. For measurements of passage saturation transfer spectra, the spectrometer was modified to detect the 2nd harmonic out-of-phase signal.

Saturation transfer spectra are sensitive to the effective microwave field strength  $H_1$ . Since measurements were made with samples containing various amounts of water and at various temperatures, and since the dielectric constant of water and its temperature dependency are very large, the incident microwave power should be readjusted to obtain comparable values of  $H_1$  for different samples at different temperatures. For this purpose, the power saturation curve for 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl powder attached on the outside of a sample tube with various contents was measured at various temperatures. The microwave power at which extrapolation of the initial slope reaches the saturation level was determined (Fig. 1). For calibration of the water content in recombinant samples, the ESR peak height of MnCl<sub>2</sub> in MgO inserted into the cavity was compared with that in the presence of reference protein-phospholipid suspensions: BSA (40 mg/ml)-egg yolk phosphatidylcholine (75 or 300 mM). The appropriate power was determined by interpolation of the two curves in Fig. 1 (○ and ●).

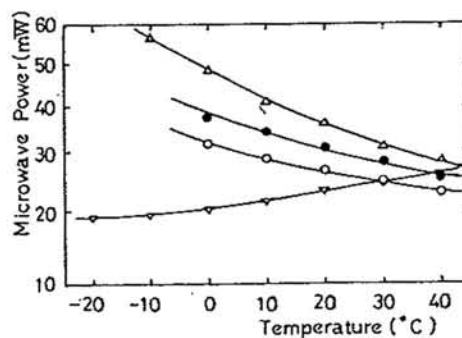


Fig. 1. Incident microwave power on the dial required to give the same effective magnetic field strength  $H_1$  on the sample. Data were obtained with 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl powder in the presence of water ( $\Delta$ ), water-glycerol at 53 : 47 molar ratio ( $\nabla$ ), and bovine serum albumin (40 mg/ml)-egg yolk phosphatidylcholine [75 mM ( $\bullet$ ) or 300 mM ( $\circ$ )]. See "MATERIALS AND METHODS." The equivalent power in the presence of air was 16 mW at 20°C.

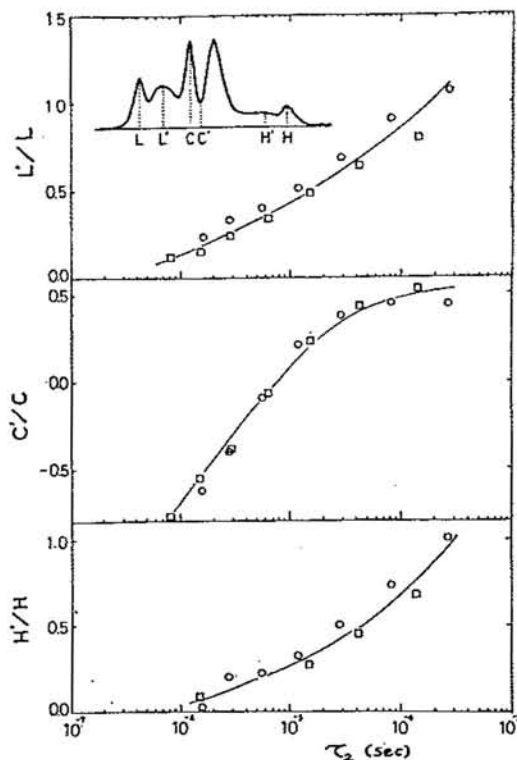


Fig. 2. Relationship between the rotational correlation time  $\tau_2$  and the peak height ratios  $L'/L$ ,  $C'/C$ , and  $H'/H$  of the saturation transfer spectrum of the maleimide-spin-labeled hemoglobin ( $\square$ ) and bovine serum albumin ( $\circ$ ). The spin-labeled protein was dissolved in glycerol-water (47:53 molar ratio). Microwave power used was determined from Fig. 1. The spectrum was recorded by the second harmonic  $90^\circ$  out-of-phase detection (modulation at 50 kHz and detection at 100 kHz).

#### Estimation of Rotational Correlation Time—

The rotational correlation time was calculated from the peak height ratios of the saturation transfer spectrum by comparison with those obtained for reference systems: maleimide-spin-labeled hemoglobin and BSA. Spin labeling of BSA was carried out by reaction with the maleimide spin-label reagent at a molar ratio of 1:1 at  $0^\circ\text{C}$  overnight. The proteins were mixed with glycerol at a molar ratio of glycerol to water of 47:53 and the saturation transfer spectrum was measured at various temperatures from  $-20^\circ\text{C}$  to  $40^\circ\text{C}$ . The rotational correlation time was calculated from the viscosity of aqueous glycerol (22) on the assumption that the radii of hemoglobin and BSA were 2.9 nm (23) and 3.6 nm (24),

respectively. The relationship between the peak height ratios and the correlation time is shown in Fig. 2. Data obtained from the two different reference systems agreed rather well.

The rotational correlation time for the spin-labeled rhodopsin was calculated from the ratio of low-field peaks ( $L'/L$ ) throughout the present paper. This choice was made only for convenience and we did not pay much attention to the absolute value. For example, the rotational motion of rhodopsin in membranes was anisotropic while the motion in the reference system should be almost isotropic (7).

## RESULTS

*Rotational Motion of Rhodopsin in Recombinant Membranes*—The conventional ESR spectrum of the maleimide-spin-labeled rhodopsin in recombinants consisted only of a strongly immobilized component with essentially no weakly immobilized component which would interfere with estimation of the rotational correlation time from the saturation transfer ESR spectra.

The saturation transfer spectrum of recombinants was measured at various temperatures around the phospholipid phase transition. The apparent rotational correlation time was calculated from the low-field peak height ratio and is plotted in Fig. 3 for DMPC recombinant. The rotational motion shows a discontinuous change at the transition temperature. However, the change was not as marked as that from complete immobilization in the solid phase to fast motion in the fluid phase. The change in the correlation time in the lipid-rich recombinant was within a value of the order of  $10^{-5}$  s. The spin labels attached at the different cysteinyl residues of rhodopsin showed virtually the same correlation time and temperature dependence ( $\square$  and  $\blacksquare$  in Fig. 3).

Increasing the relative amount of rhodopsin in the recombinant affected its rotational motion, causing more inhibition. The effect was small in the lipid-rich recombinants; compare the curve for the protein-to-lipid ratio of 1:240 with that at 1:153. The inhibition of rotational motion became large when the ratio was increased to 1:43. The rotational correlation time increased about twice in the fluid phase. This tendency

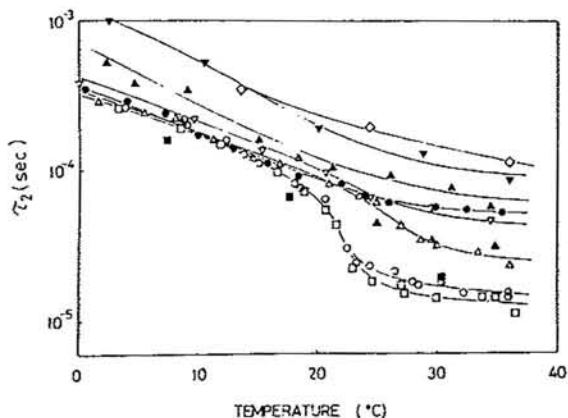


Fig. 3. Apparent rotational correlation time  $\tau_2$  of rhodopsin in DMPC recombinant with various protein-to-lipid ratios; 1 : 240 ( $\square$  and  $\blacksquare$ ), 1 : 153 ( $\circ$ ), 1 : 43 ( $\triangle$ ), and 1 : 22 ( $\bullet$ ). The spin label was attached at the cysteinyl residue in the carbohydrate-carrying segment of rhodopsin except for  $\blacksquare$ , with the spin label on the retinal-carrying segment (see the text). Results of various cross-linking treatments of recombinant (1 : 43) are also included:  $\triangle$ , rhodopsin + anti DNP-BSA antisera;  $\nabla$ , DNP rhodopsin + anti DNP-BSA antisera produced in goat;  $\blacktriangle$ ,  $\nabla$  + anti goat-IgG antisera;  $\blacktriangledown$ , glutaraldehyde treatment of  $\blacktriangle$ ;  $\diamond$ , treatment of  $\blacktriangledown$  with BSA.

continued in the recombinant with more rhodopsin (1 : 22). On the other hand, the effect on the rotational motion in the host lipid solid phase was small. Consequently, the temperature dependence for the 1 : 22 recombinant had no discontinuity at the lipid phase transition.

Cross-linking of rhodopsin inhibited the rotational motion to various extents. Glutaraldehyde treatment caused a 3-fold increase in the correlation time in the fluid phase. Further treatment with BSA caused a further increase in the correlation time (5-fold) (data not shown). The treatments did not much affect the rotational motion in the solid phase. The residual mobility after glutaraldehyde treatment may be due to the formation of long and flexible cross-bridges of glutaraldehyde polymers between the amino residues of rhodopsin molecules, since glutaraldehyde exists in the form of  $\alpha$ ,  $\beta$ -unsaturated aldehyde polymers with different degrees of polymerization (25). Further reduction of the rotational motion by the albumin treatment may be due to cross-linking of the residual aldehyde groups in the

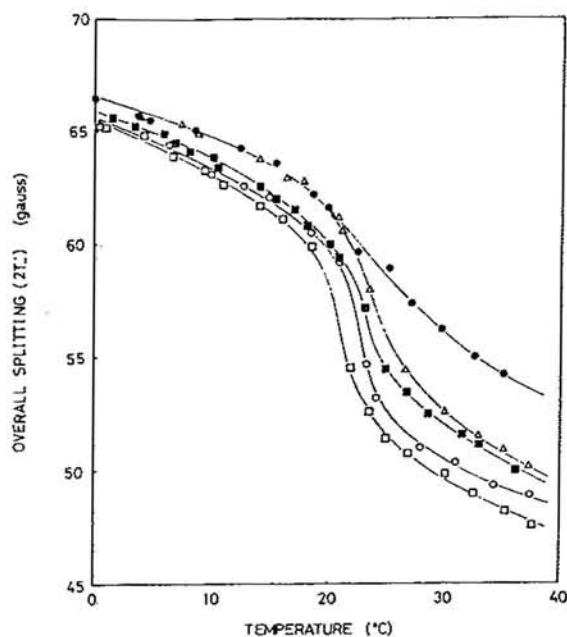


Fig. 4. Temperature dependence of the overall splitting value for 5-doxyl stearate in DMPC recombinant with various rhodopsin-to-lipid ratios: 1 : 153 ( $\circ$ ), 1 : 90 ( $\blacksquare$ ), 1 : 43 ( $\triangle$ ), and 1 : 22 ( $\bullet$ ).  $\square$ : a control without rhodopsin.

cross-bridges. Cross-linking by antibodies also caused increase in the rotational correlation time (see Fig. 3). Further cross-linking of the antibody-treated recombinants with glutaraldehyde and BSA caused strong immobilization. Some oscillatory rotational mobility still remained, however. The results of the cross-linking study show that the spin label motion reflects the rotational motion of rhodopsin as a whole in the recombinant membranes.

*Effect of Incorporation of Rhodopsin on Phospholipid*—The effect of rhodopsin on the host phospholipid was investigated at two different 'depths' using 5-doxyl and 16-doxyl stearate. Figure 4 shows the temperature dependence of the overall splitting of the conventional ESR spectrum for 5-doxyl stearate in DMPC recombinant. Rhodopsin caused an increase in the overall splitting; *i.e.*, a decrease in the lipid alkyl chain flexibility. The effect became larger with increase of the protein content in the recombinant and was larger in the fluid phase than in the solid phase. The temperature dependence profile became more

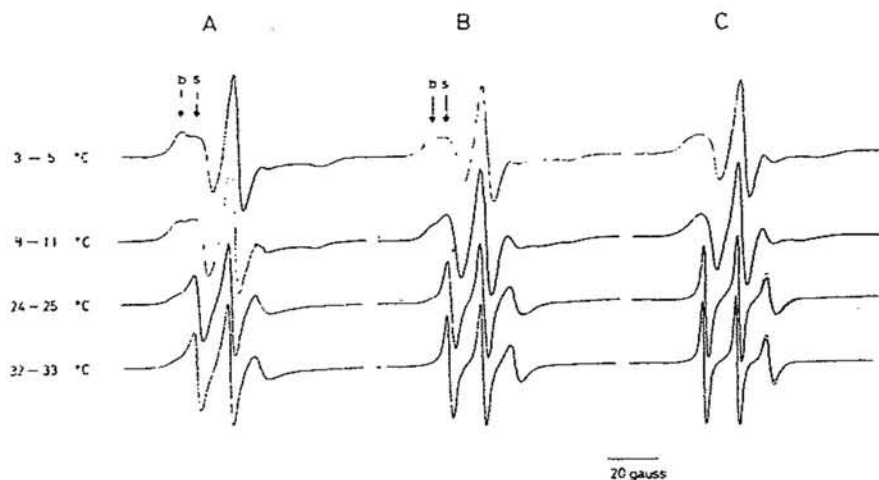


Fig. 5. Conventional ESR spectra of 16-doxyl stearate in DMPC recombinant with various rhodopsin-to-lipid ratios; (A) 1 : 27, (B) 1 : 53, and (C) 1 : 153. Spectra were measured both above and below the phospholipid phase transition temperature (23°C). Dotted lines in (C) are for pure DMPC liposomes. The two spectral components b and s are the strongly immobilized and weakly immobilized components, respectively.

and more broadened with increase of the protein-to-lipid ratio. The change in the overall splitting is not due to polarity change around the nitroxide moiety since the ESR spectrum measured at a low temperature ( $-100^{\circ}\text{C}$ ) had the same overall splitting value (69.1 G) as that for the spin label in pure DMPC liposomes, nor was it ascribable to a change in the relative population of the non-protonated form of 5-doxyl stearic acid (26).

Rhodopsin caused a broadening of ESR spectrum for 16-doxyl stearate in DMPC recombinant (Fig. 5). The effect was very small but observable in the lipid-rich recombinant (1 : 153) (compare the dotted and full line spectra in Fig. 5C). The broadening became larger with increase in the protein-to-lipid ratio. Increasing the protein concentration also caused the appearance of a strongly immobilized component (component b in Fig. 5, A and B). The relative population of the component was larger in the recombinant containing more protein. The strongly immobilized component was markedly increased below the phase transition temperature. The spin labels in the solid phase phospholipid are not as strongly immobilized since the spectrum for the lipid-rich recombinant and also that for pure DMPC liposomes were not much broadened below the phase transition temperature (see Fig. 5C).

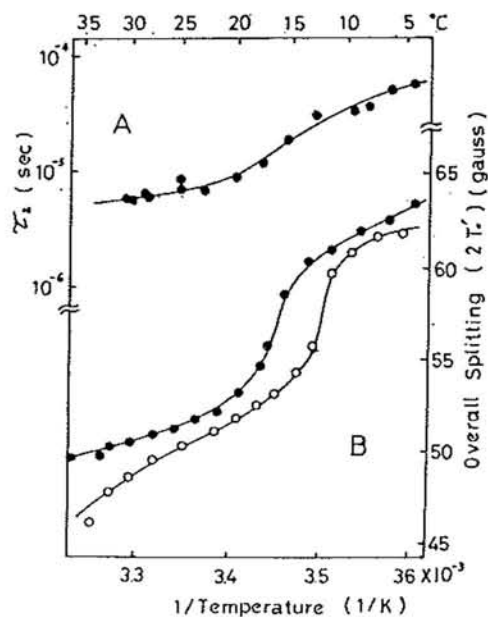


Fig. 6. Apparent rotational correlation time  $\tau_2$  of rhodopsin (A) and the overall splitting value for 5-doxyl stearate (B) in DEPC recombinant as a function of temperature. The molar ratio of rhodopsin to phospholipid was 1 : 300.  $\circ$  represents pure DEPC liposomes. Spin-labeling of rhodopsin was carried out at  $0^{\circ}\text{C}$  for 8 h at a molar ratio of the maleimide spin-label reagent to rhodopsin of 100 : 1.

*Rhodopsin-DEPC Recombinant*—The protein rotational mobility and lipid alkyl chain flexibility in the recombinant were studied for comparison with those in the DMPC recombinant. Figure 6 shows the result for a lipid-rich recombinant (1 : 300). The rotational motion of rhodopsin showed some discontinuous change at the host lipid phase transition but the change in the correlation time was not large. Rhodopsin caused a decrease in the lipid alkyl chain flexibility in the fluid phase and also in the solid phase. The effect in the fluid phase was somewhat smaller than that in the corresponding DMPC recombinant (1 : 300) (data not shown).

#### DISCUSSION

Rhodopsin caused some reduction of the lipid alkyl chain flexibility in the fluid phase in the time scale of conventional ESR,  $10^{-7}$  s. The effect was observed as an increase in the overall splitting value for 5-doxyl stearate (Fig. 4) and a broadening of the spectral line for 16-doxyl stearate (Fig. 5). The effect was larger with increase of the amount of rhodopsin in recombinants. This may be taken as an increase in the 'average' viscosity of the lipid bilayer media by rhodopsin. Similar observations have been made by other investigators (8, 10). Hong and Hubbell found a linear dependence of the order parameter on the protein-to-lipid ratio in rhodopsin recombinants (8).

Rhodopsin can reduce the lipid alkyl chain flexibility when phospholipid molecules come into contact with its hydrophobic surface. As the protein concentration increases in the recombinant, the residence time for lipids or spin labels on the protein surface will increase. The ESR spectrum to be observed should be some average or addition of the spectra due to spin labels on the protein surface and in the bulk lipid bilayer phase appropriately weighted by the residence time. In view of the exchange rate ( $10^7$  s $^{-1}$ ) of phospholipid molecules between neighboring sites in the fluid phase (27), the spectrum must have been somehow averaged. This may explain the gradual increase in the overall splitting and the line width with increasing protein-to-lipid ratio.

In the protein-rich recombinants, a strongly immobilized component appeared in the ESR spectrum for 16-doxyl stearate, in addition to the

weakly immobilized component due to spin labels in the lipid bilayer phase (Fig. 5). The exchange rate between the strongly immobilized sites and the bilayer phase must be much smaller than  $10^7$  s $^{-1}$ , since the two components were observed as separate peaks. The absence of a strongly immobilized component in the spectrum for 5-doxyl stearate may be due to large overall splitting for the spin label in the bilayer phase. Peaks due to the two components would come close to being unresolved.

The strongly immobilized component has been ascribed to spin labels bound on the hydrophobic surface of integral proteins (boundary lipids) (28, 29). In the present rhodopsin recombinant system, we prefer to interpret the component as being due to spin labels bound between the protein molecules or entrapped in the protein aggregates (entrapped lipid model). Such a model was originally put forward by Chapman *et al.* (30) for the interaction of gramicidin A with phospholipid.

The appearance of the strongly immobilized component and its increase with the protein content (Fig. 5) can be qualitatively explained by both models; in terms of increased protein-surface area by the boundary lipid model and increased protein aggregation by the entrapped lipid model. However, the large increase in the component on going down to the solid phase may be compatible only with the entrapped lipid model, since extensive protein aggregation or clustering would occur on host lipid crystallization. The boundary lipid model may not be compatible with this because the protein boundary surface would decrease on aggregation. Extensive protein aggregation in the solid phase has been demonstrated for rhodopsin-phospholipid (1 : 100) recombinants by freeze-fracture electron microscopy (9). In the fluid phase of DMPC, rhodopsin molecules were dispersed. However, in DEPC recombinants, rhodopsin molecules were partially aggregated in the fluid phase, although at higher temperature (37°C) the aggregates disappeared (9). The large increase in the strongly immobilized component in the solid phase cannot be ascribed to spin labels entrapped in the solid phospholipid, as noted in "RESULTS."

Favre *et al.* have recently investigated environments near the hydrophobic surface of rhodopsin using stearate spin labels covalently attached to the protein and found that the motion of 16-doxyl

stearate was not strongly immobilized on the surface and was only slightly reduced in the lipid-rich recombinant (12). They observed the appearance of the strongly immobilized component on delipidation or extensive photo-bleaching of disc membrane (12) or on increasing the protein content in egg yolk phosphatidylcholine recombinant (13). In a very recent independent paper (31), they assigned the strongly immobilized component to entrapped lipid in the protein aggregates, as we have done in the present paper. On the other hand, Watts *et al.* (32) have observed the strongly immobilized component in rod outer segment membrane (amounting to 33–43%) and interpreted it as being due to the boundary lipids in direct contact with the rhodopsin surface.

The rotational mobility of rhodopsin in recombinant membrane changed discontinuously at the host lipid phase transition, but the change was not substantial (Figs. 3 and 6). There was still some rotational mobility in the solid phase, which may represent oscillatory rotational motion in the lipid-sandwiched protein aggregates. In order to analyze the motion in the fluid phase, the apparent rotational correlation time was replotted against the overall splitting value for 5-doxyl stearate, which may represent the average viscosity of the lipid bilayer media. The result shows that the relationship was dependent on the protein content in the recombinant (Fig. 7). The rotational correlation time at the same viscosity was larger for the recombinant containing more rhodopsin molecules. This result can be explained by assuming protein aggregation since, if the

protein molecules are dispersed as monomers, the rotational correlation time should be determined by the average viscosity. The analysis is therefore consistent with the assignment of the strongly immobilized component to lipid spin labels entrapped in the protein aggregates. The weakened response of the rotational motion to the host lipid phase transition may thus be due to residual mobility in the protein aggregates in the solid phase on one hand, and remaining aggregation in the fluid phase on the other hand. The response became more and more weakened with increase of the protein content in recombinants (Fig. 3), probably because of increased aggregation in the fluid phase and partly because of the increased average viscosity. Slower rotational motion of protein in membranes containing more proteins has also been observed for bacteriorhodopsin (33). The authors have discussed the effect in terms of increased average viscosity in the protein-rich recombinant.

When a plot similar to Fig. 7 was made for DEPC recombinant (1 : 300), the rotational correlation time at the same viscosity was larger than that for the corresponding DMPC recombinant (data not shown). This may be due to increased aggregation of rhodopsin molecules in the fluid phase of DEPC. The smaller effect of rhodopsin on the lipid alkyl chain flexibility in DEPC may also be related to greater aggregation of the proteins. Such small but definite differences between the two kinds of phospholipids in the interaction with rhodopsin must reflect a general dependence of protein-lipid interactions on the constituents.

Finally, it would be interesting to investigate the possible correlation between the permeability properties of disc membranes and the aggregation of rhodopsin molecules.

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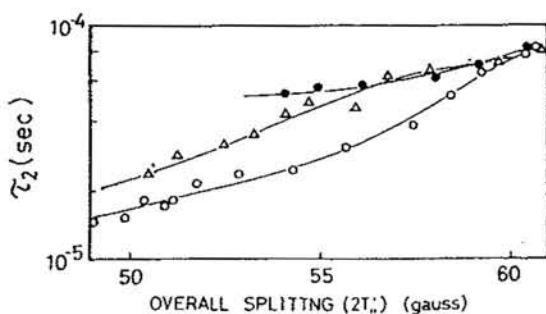


Fig. 7. Correlation between the apparent rotational correlation time of rhodopsin and the overall splitting value for 5-doxyl stearate in DMPC recombinant above the phase transition temperature. Rhodopsin-to-lipid ratio; 1 : 153 (O), 1 : 43 ( $\Delta$ ), and 1 : 22 ( $\bullet$ ).

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